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(54) Title: COMPOSITIONS AND METHODS FOR TREATING RHEUMATOID ARTHRITIS (57) Abstract <p>The present invention provides peptides, therapeutic compositions, and methods for treatment of rheumatoid arthritis in mammals, specifically in humans. The peptides of the invention comprise fragments of type II collagen which bind specifically with human major histocompatibility complex proteins known to be genetically linked to susceptibility to rheumatoid arthritis. The therapeutic compositions of the invention comprise the peptides, alone or in combination with other collagen peptides. In one method of the invention, therapeutic compositions of the peptides are administered by injection, transdermally, by inhalation, or orally to a patient in need of treatment.</p>		

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COMPOSITIONS AND METHODS FOR TREATING RHEUMATOID ARTHRITIS

BACKGROUND OF THE INVENTION

Rheumatoid arthritis (RA) is a chronic multisystem disease whose common clinical manifestation is persistent inflammatory synovitis of the peripheral joints resulting in proliferation of synovial cells and subsequent pannus formation, cartilage destruction, bone erosion, and ultimately joint deformity and loss of joint function. Joints containing articular cartilage of which type II collagen is a major component are particularly affected. RA is accompanied by joint swelling, inflammation, stiffness and pain especially upon flexing. In the advanced stages of arthritis, debilitating pain may result from even a slight movement of the joints. RA affects approximately 1% of the population of the United States and Europe as well as 0.2 to 0.4% of the Japanese population, with women being affected about three times more often than men. The prevalence of the disease increases with age, with the onset being most frequent during middle age.

Thus far, treatment of RA has been speculative, focusing on relief of symptoms of the disease and not on its cause, which is believed to be an abnormal autoimmune response to normal joint proteins. Generally, the drugs used to treat RA thus far are somewhat toxic and have failed to demonstrate a consistent advantage of one over the other. Furthermore, none of these drugs have been demonstrated to alter the course of the disease.

For example, salicylic acid and other nonsteroidal anti-inflammatory drugs, such as fenoprofen, ibuprofen, indomethacin, naproxen, meclofenamate, piroxicam, sulindac, tolmetin, and simple analgesics have been used to control symptoms of the inflammatory process, but have not been successful in arresting the progression of RA. The symptoms of RA have also been treated with glucocorticoids such as the steroid compounds prednisone and methylprednisolone (which are also non-specific

immunosuppressive and anti-inflammatory drugs). Steroids are known to have significant toxic side effects associated with their long term use. Disease-modifying drugs such as gold compounds, Dpenicillamine, and antimalarials have also been used in combination with nonsteroidal anti-inflammatory drugs for analgesic and anti-inflammatory effects.

Cytotoxic immunosuppressive drugs such as Imuran (azathioprine), methotrexate, cyclophosphamide, and cyclosporin A have also been used to treat RA symptoms. These drugs suppress the entire immune system and are incapable of selectively suppressing the specific abnormal autoimmune response which causes RA. Use of such non-specific immunosuppressant drugs over time increases the risk of infection, and prolonged therapy with these nonspecific cytotoxic immunosuppressive drugs entails toxic side effects, including an increased tendency toward development of certain malignancies, kidney failure, diabetes and liver function disorders. Moreover, cytotoxic immunosuppressive drug therapy merely slows down the progress of the disease, which resumes at an accelerated pace after the therapy is discontinued. For example, about six weeks after such a drug is discontinued, the patient deteriorates to the same stage of RA that existed before the treatment was begun. In addition, effectiveness of these drugs is self-limiting; they gradually cease being effective after about two to five years.

Thus, current treatments for RA are of limited efficacy, involve significant toxic side effects, and cannot be used indefinitely. New therapeutic methods of treating RA are needed which do not result in immunodeficiency, and whose long term effects are not toxic to the subject being treated. In addition, such therapeutic methods should not only reduce or alleviate symptoms, but additionally should specifically block the progression of the disease. Also, there is a need for improved methods of diagnosing RA at an early stage when damage to the joint is not great, thereby enabling the application of therapies which can halt the progression of the disease.

RA is a cell-mediated autoimmune disease, i.e., the immune system mistakenly perceives the body's own tissue as foreign and mounts an abnormal immune response against it. As with other autoimmune diseases, susceptibility to RA is believed to be linked to expression of specific class II alleles of the major histocompatibility complex (MHC): in humans, the genes encoding HLA DRB1*0101 and DRB1*0401 are linked both to inherited susceptibility to RA and to severity of RA. In mice, the genes encoding the I-Aq and I-Ar MHC proteins are linked to susceptibility to collagen-induced arthritis. However, the molecular basis of this linkage is not understood for any mammal.

As mentioned above, RA particularly affects joints containing articular collagen which in turn contains type II collagen. A substantial percentage of RA-afflicted humans possess T cells of the CD4+ type which are specifically reactive with collagen and/or have an abnormal humoral response against collagen, the most common protein in the structural support of the human or mammalian body. Collagen's basic elemental unit is the tropocollagen protein, which is composed of three polypeptide chains of the same size, wound about each other forming a superhelical cable or a triple-stranded helical rod. Each of the three chains in tropocollagen consists of about a thousand amino acid residues. At least part of RA etiology appears to derive from autoimmunity to type II collagen.

Experimental models of RA have been developed by immunizing genetically susceptible lower animals with heterologous type II collagen, or with fragments of heterologous type II collagen to elicit collagen-induced arthritis. The specific 26 amino acid peptide (26mer) fragment of type II collagen set forth in SEQ ID NO:1 has been shown both to induce arthritis in susceptible mice and to be an effective injected tolerogen for collagen-induced arthritis in these mice (L.K. Meyers et al., *J. Exp. Med.* 170:1999-2010 (1989)). In rats, however, the immunodominant epitope of type II collagen for induction of arthritis is a different portion of the molecule, and the peptide of SEQ ID NO:1 does not stimulate lymphokine production in collagen-specific rat T cell hybridomas (G. Ku et al., *Eur. J. Immunol.* 23:591-599 (1993)).

The differences between immunodominant type II collagen epitopes among species remain unexplained.

Using a mouse T cell proliferation assay, the eight amino acids corresponding to amino acids 16 to 23 of SEQ ID NO:1 were identified as the immunodominant T cell binding motif for the collagen-induced arthritis susceptibility allele of DBA/1J mice in D.D. Brand, et al., *J. Immunol.* 152:3088-3097 (1994). However, a synthetic 26mer containing the proposed immunodominant peptide binding motif did not induce arthritis. Id. at 3095.

Attempts to tolerize susceptible animals with the type II collagen fragment of SEQ ID NO:1 prior to the induction of arthritis have met with some success. As mentioned above, the 26mer fragment of SEQ ID NO:1 is an effective injected tolerogen for collagen-induced arthritis in susceptible mice. However, in such animal models, efficacy of the injected toleragen for treatment of pre-existing arthritis has not been shown.

PCT/US93/09113 discloses that oral administration of purified water-soluble whole chick type II collagen to human RA patients resulted in improvement of arthritis symptoms. Several type II collagen-derived peptides, including the peptide of SEQ ID NO:1, were orally administered to rats prior to induction of arthritis, and some suppression of arthritic symptoms was purported to occur, as compared to control animals which did not receive the peptides. PCT/US93/09113 does not disclose type II collagen-derived peptides tested had any efficacy in humans.

SUMMARY OF THE INVENTION

The present inventors have for the first time demonstrated a type II collagen peptide that binds to the human HLA DRB1*0401 major histocompatibility complex protein and comprises a T cell epitope of human type II collagen. This epitope is the immunodominant determinant recognized by T cell hybridomas generated from transgenic mice expressing the human DRB1*0401 and CD4 genes, which hybridomas

are specific for type II collagen. This immunodominant sequence is conserved in both bovine and human type II collagen proteins.

5 In one embodiment, the invention provides a peptide comprising an amino acid sequence set forth in SEQ ID NO:2, as shown in Fig. 6. In another embodiment the invention provides related peptides having the amino acid sequence set forth in SEQ ID NO:3, and the amino acid sequence set forth in SEQ ID NO:4, all as shown in Fig. 6.

10 In another embodiment, the invention provides a therapeutic composition comprising at least one peptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:2, the amino acid sequence set forth in SEQ ID NO:3, and the amino acid sequence set forth in SEQ ID NO:4 all as shown in Fig. 6, and a pharmaceutically acceptable carrier or
15 diluent. The therapeutic compositions of the invention may further provide at least one peptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:1, the amino acid sequence set forth in SEQ ID NO:5, the amino acid sequence set forth in SEQ ID NO:6, and the amino acid sequence set forth in SEQ ID NO:7, all as shown in Fig. 7.

20 In another embodiment, the invention provides a method of treating a mammal suffering from rheumatoid arthritis which comprises administering a therapeutic composition to said mammal, said therapeutic composition containing at least one peptide comprising an amino acid sequence selected from the group consisting of the
25 amino acid sequence set forth in SEQ ID NO:2, the amino acid sequence set forth in SEQ ID NO:3, and the amino acid sequence set forth in SEQ ID NO:4. The therapeutic composition administered in accordance with the invention may further comprise at least one peptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:1, the amino
30 acid sequence set forth in SEQ ID NO:5, the amino acid sequence set forth in SEQ ID NO:6, and the amino acid sequence set forth in SEQ ID NO:7.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1 sets forth the amino acid sequence of bovine type II collagen, when aligned with human type II collagen in CCG program (data not shown), bovine type II collagen has 96.434% similarity and 93.462% identity with human type II collagen (i.e. the proteins are highly conserved throughout), the human protein has a significantly longer amino terminus than does the bovine type II collagen so that residues 276-288 of bovine type II collagen (SEQ ID NO:2) is equivalent to amino acid residues 392-404 in human type II collagen.

FIG. 2A and 2B demonstrate that the collagen-specific T cell hybridomas generated from transgenic mice expressing the human DRB1*0401 and CD4 genes were restricted for human MHC determinants.

FIG. 3 shows that the peptides of SEQ ID NO:2 (collagen 276-288) and SEQ ID NO:3 (collagen 273-285) stimulate interleukin-2 secretion by the collagen-specific T cell hybridoma B210 generated from transgenic mice expressing the human DRB1*0401 and CD4 genes. The individual points are averages of four separate measurements.

FIG. 4A and 4B compare the amount of interleukin-2 secretion by hybridoma B210 when incubated with a set of peptides of varying length within the peptide of SEQ ID NO:2 (collagen 276-288). The individual points are averages of four separate measurements.

FIG. 5 demonstrates that binding of the peptide of SEQ ID NO:2 to hybridoma B210 occurs via the human HLA-DRB1*0401 protein and not via the other human MHC class II proteins DRB1*0402, DRB1*0403, DRB1*0405, or DRB1*0101. The individual points are averages of four separate measurements.

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FIG. 6 sets forth the amino acid sequences of the peptides of the invention (SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4).

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— FIG. 7 sets forth the amino acid sequences of the peptides of the invention (SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7). three and six separate measurements. Shaded amino acids differ between bovine and murine (last column) type II collagen.

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FIG. 8 shows the predicted and experimentally determined IC_{50} values for a set of peptides from bovine type II collagen binding to DRB1*0401. The peptides are listed in descending order. The experimentally determined values are averages of between three and six separate measurements. Shaded amino acids differ between bovine and murine (last column) type II collagen.

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FIG. 9 shows the measured IC_{50} values under two incubation conditions for a set of peptides defining important residues in the region of 273-288 (SEQ ID NO:4) of bovine type II collagen. Shaded amino acids could be postranslationally modified in the intact protein.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Applicants have for the first time discovered that peptides as set forth in SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, all as shown in Fig. 6, derived from human type II collagen mediate the autoimmune response characteristic of RA. Binding of the peptide of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4 to human MHC proteins on the surface of an antigen presenting cell stimulates the relevant T cell subpopulations responsible for the autoimmune response in RA and thus are believed to comprise at least one T cell epitope. A T cell epitope is the basic or smallest unit of recognition by a T cell receptor where the epitope comprises amino acids essential to T cell receptor recognition. T cell epitopes are thought to trigger early events at the level of the T helper cell by binding an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. Without being bound to any theory, these events are believed to lead to T cell proliferation, lymphokine secretion, local inflammatory reactions, recruitment of additional immune cells to the site, and activation of the B cell cascade leading to production of autoantibodies against human type II collagen, resulting in the clinical symptoms of RA.

It is believed, therefore, that administration of a therapeutic composition containing any one of the epitope-containing peptides of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, in non-immunogenic form, will alleviate the symptoms of RA in humans. While the exact mechanism by which such alleviation of RA symptoms is not known, and without wishing to be bound by any theory, peptides of the invention may act in any one or more of the following ways. Administration of any one or more of the peptides of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, in non-immunogenic form may cause T cell nonresponsiveness of appropriate T cell subpopulations, such that they become unresponsive to the type II collagen autoantigen and do not participate in stimulating an immune response upon further exposure to the type II collagen autoantigen (i.e., thereby down-regulating the immune response). Alternatively, administration of any one or more of the peptides of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4 may modify the lymphokine

secretion profile as compared with exposure to the naturally occurring type II collagen autoantigen (e.g., resulting in an increase of interleukin-4 and/or interleukin-10 resulting in an increase in interleukin-2 and/or interleukin-12).

Another mechanism by which administration of a therapeutic composition of any one or more of the peptides of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4 may act is by causing T cell subpopulations which normally participate in the response to the type II collagen autoantigen to be drawn away from the sites of exposure (e.g., the affected joints) toward the sites of administration of the composition. This redistribution of T cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the pathogenic immune response within the affected joints resulting in diminution in RA symptoms. Moreover, administration of the peptides of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4 may cause induction of T suppressor cells directly or indirectly (e.g. via a bystander antigen). The peptides of the invention may act by any one of these mechanisms or by a combination of these mechanisms.

The peptides of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, when administered to an individual suffering from RA, are capable of modifying the B cell response, the T cell response, or both the B cell and the T cell response of the individual to the type II collagen autoantigen, or can be shown to result in a diminution of symptoms. As referred to herein, a diminution of symptoms includes any reduction in joint swelling, inflammation, stiffness and pain, including pain upon flexing following a treatment regimen with a peptide of the invention. This diminution in symptoms may be determined subjectively or clinically. The isolated peptides of the invention can also be used in methods of diagnosing, treating, and preventing RA responses. Furthermore, the isolated peptides of the invention can be used in screening for RA and for developing candidates for therapeutic compositions.

The peptides of the invention may be embodied as isolated and purified peptides having the amino acid sequences set forth in SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 all as shown in Fig. 6, or as modified forms of the peptides of

SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4. The terms isolated and purified are used interchangeably herein and refer to peptides, protein, protein fragments, which are 1) substantially free of cellular material or culture medium when produced by recombinant DNA techniques, 2) substantially free of chemical precursors or other chemicals when chemically synthesized or 3) substantially free of enzymes, chemicals and other reagents when produced by chemical or enzymatic cleavage of the full length protein. Accordingly, an isolated peptide may be produced recombinantly, synthetically (such as by chemical synthesis) or by chemical or enzymatic cleavage of the whole or partial protein, and is substantially free of all cellular material and culture medium or substantially free of chemical precursors or other chemicals and enzymes.

In accordance with the present invention, the peptides of SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 may be modified for such purposes as increasing solubility (particularly desirable if the composition is to be injected), enhancing therapeutic or preventive efficacy, or stability (e.g., shelf life ex vivo, and resistance to proteolytic degradation in vivo). A modified peptide within the scope of the present invention can be produced in which the amino acid sequence of peptides set forth in SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 has been altered. It is preferred that a modified peptide of the invention is capable of eliciting a T cell response such as T cell stimulation (e.g. proliferation or lymphokine secretion) and/or is capable of inducing T cell non-responsiveness in appropriate T cell populations which participate in the RA autoimmune response. Modification may include for example, amino acid substitution, deletion, or addition. The peptides of the invention may further be modified by extension at the N-and/or C-terminus with irrelevant amino acid sequence. In addition, amino acid side chains of peptides of the invention can be chemically modified or by cyclization of the peptide.

For example, a peptide can be modified so that it maintains the ability to induce T cell non-responsiveness or reduced T cell responsiveness and bind MHC proteins without the ability to induce a strong proliferative response or possibly, and proliferative response when administered in immunogenic form. In this instance,

critical binding residues for the T cell receptor can be determined using known techniques (e.g., substitution of each residue and determination of the presence or absence of T cell reactivity). Those residues shown to be essential to interact with the T cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance, diminish but not eliminate or not affect T cell activity. In addition, those amino acid residues which are not essential for T cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance, diminish but not eliminate or not affect T cell activity but does not eliminate binding to relevant MHC.

Additionally, peptides of the invention can be modified by replacing an amino acid shown to be essential to interact with the MHC protein complex with another, preferably similar amino acid residue (conservative substitution) whose presence is shown to enhance, diminish but not eliminate or not affect T cell activity. In addition, amino acid residues which are not essential for interaction with the MHC protein complex but which still bind the MHC protein complex can be modified by being replaced by another amino acid whose incorporation may enhance, not affect, or diminish but not eliminate T cell reactivity. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine, glutamic acid, or a methyl amino acid.

In order to enhance stability and/or reactivity, peptides of the invention can also be modified to incorporate one or more polymorphisms in the amino acid sequence of the protein allergen resulting from natural allelic variation.

Additionally, D-amino acids, non-natural amino acids or non-amino acid analogues can be substituted or added to produce a modified protein or peptide within the scope of this invention. Furthermore, peptides of the present invention can be modified using the polyethylene glycol (PEG) method of A. Schon and co-workers (Wie et al., (1981) *Int. Arch. Allergy Appl. Immunol* 64:84-99) to produce a protein or peptide conjugated with PEG. In addition, PEG can be added during chemical synthesis of a protein or peptide of the invention. Modifications of proteins or

peptides or portions thereof can also include reduction/ alyklation (Tarr in: *Methods of Protein Microcharacterization*, J.E. Silver ed. Humana Press, Clifton, NJ, pp 155-194 (1986)); acylation (Tarr, *supra*); chemical coupling to an appropriate carrier (Mishell and Shiigi, eds, *Selected Methods in Cellular Immunology*, WH Freeman, San Francisco, CA (1980); U.S. Patent 4,939,239; or mild formalin treatment (Marsh *International Archives of Allergy and Applied Immunology*, 41:199-215 (1971)).

To facilitate purification and potentially increase solubility of proteins or peptides of the invention, it is possible to add reporter group(s) to the peptide backbone. For example, poly-histidine can be added to a peptide to purify the peptide on immobilized metal ion affinity chromatography (Hochuli, E. et al., *Bio/Technology*, 6:1321-1325 (1988)). In addition, specific endoprotease cleavage sites can be introduced, if desired, between a reporter group and amino acid sequences of a peptide to facilitate isolation of peptides free of irrelevant sequences.

In order to successfully desensitize an individual to a peptide, it may be necessary to increase the solubility of a peptide for use in buffered aqueous solutions, such as pharmaceutically acceptable carriers or diluents, by adding functional groups to the peptide, terminal portions of the peptide, or by not including hydrophobic T cell epitopes or regions containing hydrophobic epitopes in the peptides or hydrophobic regions of the protein or peptide. For example, to increase solubility, charged amino acids or charged amino acid pairs or triplets may be added to the carboxy or amino terminus of the peptide. Examples of charged amino acids include, but are not limited to arginine (R), lysine (K), histidine (H), glutamic acid (E), and aspartic acid (D). Removal of hydrophobic amino acid residues from either the carboxy or amino terminus of the peptide prior to the addition of charged amino acids is also contemplated.

To potentially aid proper antigen processing of T cell epitopes within a peptide, canonical protease sensitive sites can be recombinantly or synthetically engineered between regions, each comprising at least one T cell epitope. For

example, charged amino acid pairs, such as KK or RR, can be introduced between regions within a peptide during recombinant construction of the peptide. The resulting peptide can be rendered sensitive to cathepsin and/or other trypsin-like enzymes cleavage to generate portions of the peptide containing one or more T cell epitopes.

Site-directed mutagenesis of DNA encoding a peptide or protein of the invention can be used to modify the structure of the peptide or protein by methods known in the art. Such methods may, among others, include PCR with degenerate oligonucleotides (Ho et al., *Gene*, 77:51-59 (1989)) or total synthesis of mutated genes (Hostomsky, Z. et al., *Biochem. Biophys. Res. Comm.*, 161:1056-1063 (1989)). To enhance bacterial expression, the aforementioned methods can be used in conjunction with other procedures to change the eucaryotic codons in DNA constructs encoding protein or peptides of the invention to ones preferentially used in *E. coli*, yeast, mammalian cells, or other eukaryotic cells.

Modified peptides of the invention which maintain similar antigenicity (i.e. T cell reactivity) of the parent from which the modified peptide was derived, are preferred. Human T cell stimulating activity of the peptides within the scope of the invention can be tested by culturing T cells obtained from a subject having RA with a human type II collagen peptide as set forth in SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4 and determining whether proliferation of T cells occurs in response to the peptide as measured, e.g., by cellular uptake of tritiated thymidine. Stimulation indices for responses by T cells to peptides of the invention can be calculated as the maximum counts per minute (CPM) in response to a peptide divided by the control CPM. A stimulation index (S.I.) equal to or greater than two times the background level is considered "positive". Positive results are used to calculate the mean stimulation index for each peptide for the group of patients tested. Preferred peptides of this invention comprise at least one T cell epitope and have a mean T cell stimulation index of greater than or equal to 1.5. A peptide having a mean T cell stimulation index of greater than or equal to 1.5 in a significant number of patients tested (i.e., at least 10% of patients tested) is considered useful as a therapeutic

agent. Preferred peptides have a mean T cell stimulation index of at least 1.5, more preferably at least 2.0 to 3.0.

5 Preferred modified peptides within the scope of the invention can also be identified by their ability to effect a relatively higher frequency of T cells in a patient as is described in the Examples. This frequency is measured by generating multiple identical cultures from one patient with limiting numbers of lymphocytes and a human type II collagen peptide of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID
10 —NO:4. Individual cultures are analyzed for positive reactivity with a peptide, as defined by stimulation index (described above). The frequency of peptide-reactive T cells is the percentage of cultures from the patient that show a positive stimulation index.

15 When testing peptides or modified peptides of the invention using T cell stimulating assays it is preferred that peptides useful for therapeutic purposes have a positivity index (P.I.) of at least about 100, more preferably at least about 200 and most preferably at least about 300. The positivity index for a peptide is determined by multiplying the mean T cell stimulation index by the percent of individuals, in a population of RA patients (e.g., preferably at least 15 individuals, more preferably
20 at least 30 individuals or more), who have a T cell stimulation index to such peptide of at least 1.5, more preferably at least 2.0. Thus, the positivity index represents both the strength of a T cell response to a peptide (S.I.) and the frequency of a T cell response to a peptide in a population of autoimmune individuals.

25 In accordance with the invention, an effective amount of any of the peptides described above is defined as an amount sufficient to cause down-regulation of the RA autoimmune response. Down-regulation of the RA autoimmune response in humans may be determined clinically whenever possible, or down-regulation may be determined subjectively (i.e. the patient feels as if some or all of the RA symptoms
30 have been alleviated). Effective amounts of the therapeutic compositions of the invention will vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, the ability of the peptide to

elicit an antigenic response in the individual and idiosyncratic responses of the individual.

The present invention is also embodied as a therapeutic composition
5 comprising an effective amount of at least one isolated peptide having an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:2, the amino acid sequence set forth in SEQ ID NO:3, and the amino acid sequence set forth in SEQ ID NO:4, said amino acid sequences also being shown in Figure 6. In addition to compositions containing at least one of the
10 above-referenced peptides, mixtures of at least two peptides (e.g., a physical mixture of at least two peptides), each comprising at least one T cell epitope of type II collagen may also be included in the therapeutic composition of the invention. As examples of such mixtures, the therapeutic compositions of the invention may include, in addition to at least one peptide of SEQ ID NO:2, SEQ ID NO:3, or SEQ
15 ID NO:4, all as shown in Fig. 6, one or more of the peptides shown in Figure 7 (SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7) A therapeutically effective amount of one or more of the above compositions can be administered simultaneously or sequentially, in therapeutic form to an individual suffering from RA.

20 In addition a therapeutic composition of the invention may include at least one pharmaceutically acceptable carrier or diluent. As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, absorption
25 delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional medium or agent is incompatible with the active compound (i.e., the peptides of the invention), use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the
30 therapeutic compositions of the invention. The peptides of the invention may further be administered to an individual in an appropriate diluent, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. Pharmaceutically

acceptable diluents include saline and aqueous buffer solutions. Enzyme inhibitors include pancreatic trypsin inhibitor, diethylpyrocarbonate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al., (1984) *J. Neuroimmunol.* 7:27).

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For purposes of inducing T cell nonresponsiveness, the therapeutic composition is preferably administered in non-immunogenic form, e.g., one that does not contain adjuvant. While not intending to be limited to any theory, it is believed that T cell non responsiveness or reduced T cell responsiveness is induced as a result of not providing a "second signal". Briefly, it is believed that stimulation of T cells requires two types of signals, the first is the recognition by the T cell via the T cell receptor of appropriate MHC-associated processed antigens on antigen presenting class (APCs) and the second type of signal is referred to as a "second signal" or "costimulatory signals" which may be provided by certain competent APCs. When a composition of the invention is administered without adjuvant, it is believed that competent APCs which are capable of producing the second signal or costimulatory signal are not engaged in the stimulation of appropriate T cells therefore resulting in T cell non responsiveness or reduced T cell responsiveness. In addition, there are a number of antibodies or other reagents capable of blocking the delivery of costimulatory signals such as the "second signal" which include, but are not limited to B7 (including B7-1, B7-2, and BB-1), CD28, CTLA4, CD40 CD40L CD54 and CD11a/18 (Jenkins and Johnson, *Current Opinion in Immunology*, 5:361-367 (1993), and Clark and Ledbetter, *Nature*, 367:425-428 (1994)) Thus, a peptide of the invention may be administered in nonimmunogenic form as discussed above, in conjunction with a reagent capable of blocking costimulatory signals such that the level of T cell nonresponsiveness is enhanced.

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Another aspect of this invention pertains to a multi-peptide formulation suitable for pharmaceutical administration. The multi-peptide formulation includes at least two or more peptides of the invention. Special considerations when preparing a multi-peptide formulation include maintaining the solubility and stability of all peptides in the formulation in an aqueous solution at a physiologically acceptable pH

(e.g. pH4-pH9 and even more preferably pH5.5 to pH8.5). This requires choosing one or more pharmaceutically acceptable solvents and excipients which are compatible with all the peptides in the multi-peptide formulation. For example, suitable excipients include sterile water, sodium phosphate, mannitol or both sodium phosphate and mannitol. An additional consideration in a multi-peptide formulation is the prevention of dimerization of the peptides if necessary. Agents may be included in the multi-peptide formulation which prevent dimerization, such as EDTA, or any other material or procedures known in the art to prevent dimerization. In addition pharmaceutically acceptable counter ions may be added during the preparation of the multi-peptide formulation. Examples of pharmaceutically acceptable counter ions include acetate, HCl and citrate.

The peptides of the invention, or therapeutic compositions thereof, may be administered in any convenient manner such as by injection (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like), oral administration, sublingual administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, composition may include a coating with a material to protect from the action of enzymes, acids and other natural conditions which may cause inactivation. For administration by injection, for example, about 1 μ g to about 3mg and preferably from about 20 μ g to about 1.5 g of protein or peptide per dosage unit is typical. Therapeutic compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. The injectable therapeutic composition of the invention must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabenz, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. The pharmaceutically acceptable carrier for the injectable therapeutic composition of the invention can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for

example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity of the injectable composition can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion
5 and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the injectable therapeutic composition of the invention. Prolonged absorption of the injectable compositions can be accomplished by including in the
10 composition an agent which delays absorption, for example, aluminum monostearate, gelatin or the like.

Sterile injectable solutions can be prepared by incorporating the active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered
15 sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the
20 active ingredient (i.e., the peptide or peptides of the invention) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

For subcutaneous injection of one or more therapeutic compositions of the invention, preferably about 1 μ g- 3 mg and more preferably from about 20 μ g-1.5
25 mg, and even more preferably about 50 μ g- 750 mg of each active component (peptide) per dosage unit may be administered. It is especially advantageous to formulate parenteral compositions in unit dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suited as unitary dosages for human subjects to be treated; each unit containing
30 a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the desired pharmaceutical carrier. The specifications for the novel unit dosage forms of the invention are dictated by and

directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of human subjects.

5 To administer a composition of the invention by other than parenteral administration, (i.e. oral administration) it may be necessary to coat the composition with, or co-administer the composition with, a material to prevent its inactivation or enhance its absorption and bioavailability. For example, a peptide formulation may be co-administered with enzyme inhibitors such as those mentioned above, or in
10 liposomes as described above. When the peptide of the invention is suitably protected, the protein or peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The protein and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration,
15 the peptide of the invention may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. In addition, the active compound may be incorporated into sustained-release or controlled release (steady state or pulsatile release) preparations and formulations. Such compositions and preparations contain an
20 effective amount of the peptide of the invention. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit contains between from about 10 μ g to about 200 mg of active compound. The percentage of the composition and preparations
25 may, of course, be varied and may conveniently be between about 5% to about 80% of the weight of the unit. A therapeutically effective amount of one or more of such compositions can be administered simultaneously or sequentially.

30 A preferred therapeutic/prophylactic treatment regimen with the human type II collagen peptides of the invention (which results in prevention of, or delay in, the onset of RA disease symptoms or results in reduction, progression, or alleviation of RA symptoms, i.e., down-regulation of a specific RA autoimmune response)

comprises administration, in non-immunogenic form (e.g., without adjuvant) of an effective amount of at least one peptide of the invention. Administration of the therapeutic compositions to an individual, in a non-immunogenic form, can be carried out as described above using known procedures at dosages and for periods of time effective to ameliorate the disease, i.e., to cause down regulation of the specific RA autoimmune response, thus reducing the RA symptoms of the individual.

In another aspect of the invention, individuals suffering from RA may be treated by subcutaneous administration, in non-immunogenic form, of at least one or more peptides from the following group of peptides: the amino acid sequence represented in SEQ ID NO:1, the amino acid sequence represented in SEQ ID NO:5, the amino acid sequence represented in SEQ ID NO:6, the amino acid sequence represented in SEQ ID NO:7.

Dosage regime may be adjusted by the attending physician to provide the optimum therapeutic response. For example, several divided doses may be administered over the course of days, weeks, months or years, or the dose may be proportionally increased or reduced with each subsequent injection as indicated by the exigencies of the therapeutic situation. In one preferred therapeutic regimen, subcutaneous injections of therapeutic compositions are given once a week for 3-6 weeks. The dosage may remain constant for each injection or may increase or decrease with each subsequent injection. A booster injection may be administered at intervals of about three months to about one year after initial treatment and may involve only a single injection or may involve another series of injections similar to that of the initial treatment.

The peptides of the present invention can be used in isolated and purified form for standardization of reagents for the diagnosis and treatment of RA. The isolated and purified peptide is also useful to prepare antisera or monoclonal antibodies for use in diagnosis. An animal such as a mouse or rabbit can be immunized with an immunogenic form of the isolated peptide, if necessary,

conferring immunogenicity on the peptide by coupling to carriers or by other techniques well known in the art. The peptide can be administered in the presence of adjuvant, and progress of immunization can be monitored by detection of antibody titers in plasma or serum by standard ELISA or other immunoassay methods which employ the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and polyclonal antibodies isolated, if desired, from the serum. To produce monoclonal antibodies, antibody producing cells (lymphocytes) are harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Hybridoma cells can be screened immunochemically for production of antibodies reactive with the peptides of the invention. The antisera or monoclonal antibodies can be used to standardize reagents in standard assays.

Peptides of the present invention or antibodies specific thereto can also be used for detecting and diagnosing RA. For example, this could be done by combining blood, or blood products, obtained from an individual with an isolated antigenic peptide under conditions appropriate for binding of components in the blood (e.g., antibodies, HLA molecules, T cells and B cells) with the peptide(s) or protein, and determining the extent to which such binding occurs. Other diagnostic methods for RA which the peptides of the present invention or antibodies thereto can be used include paper radioimmunosorbent test (PRIST), enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA), and luminescence immunoassays (LIA).

The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be employed to obtain similar results.

EXAMPLE I

HUMAN DRB1*0401 T CELL HYBRIDOMAS

Two DRB1*0401 transgenic mice (L. Fugger, et al., *Proc Natl. Acad. Sci. USA* 91: 6151 (1994)) were immunized by injection at the base of the tail with 100 μ l of bovine type II collagen (Sigma) dissolved in 0.1 M acetic acid. Ten days later the draining inguinal and popliteal lymph nodes were dissected and the resultant lymphocytes were stimulated in vitro with intact type II collagen. The lymphocytes were fused 48 hours later with the TCR α - β -variant of BW5147 cell line and plated out in ten 96 well plates.

A mixture of bovine type II collagen cyanogen bromide fragments was generated as follows. Bovine type II collagen (Sigma, St. Louis, MO) (10 mg) was combined with 5 mg of cyanogen bromide (Aldrich) and dissolved in 5 ml of formic acid. After incubation at room temperature overnight, the acid was diluted with water, and the liquid removed by lyophilization. The resultant mixture of cleavage products was dissolved in 10 ml of phosphate buffered saline pH 7.4. The reaction was shown to have gone to completion by analysis of the mixture on a C-4 reverse phase HPLC column.

Ten days after the fusion described above, the positive wells were transferred to 48 well plates, expanded, and assayed for interleukin-2 production in the presence or absence of the mixture of cyanogen bromide fragments of type II collagen (100 μ g/ml). T cell hybridomas (2×10^4 cells/well) were incubated with 5×10^4 antigen presenting cells, either transgenic murine spleen cells or a DRB1*0401 homozygous B cell line (Priess), in U-bottomed 96 well microtiter plates with Iscove's modified Dulbecco's medium containing 10% fetal calf serum, penicillin, streptomycin, 5×10^{-5} M β -mercaptoethanol. Supernatants from each well (100 μ l) were transferred to duplicate 96 well plates containing the interleukin-2 dependent cell line (CTLL) (5×10^3 cells/well). After 12 hours, 5 μ l of alamar blue (Alamar Biotech, Sacramento, CA) was added to each well and the

concentration of interleukin-2 was quantified by measuring the absorption at 565 nm minus that at 595 nm.

Using the procedure set forth above, a set of thirty CD4⁺ T cell hybridomas specific for bovine type II collagen were generated. In addition to expressing the human MHC protein DRB1*0401, the immunized mice also expressed the murine MHC protein I-A^f. It was necessary therefore to confirm that the T cell hybridomas were restricted for the human and not the murine antigen. Of the thirty CD4⁺ T cell hybridomas specific for type II collagen, 28 (93%) appeared to be restricted by the human MHC protein in this screening assay, responding to the human antigen presented either by the spleen cells or the B cell line. Only two clones, A37 and B96, responded to the spleen cells and not to the DRB1*0401 positive B cell line, indicating that these two clones were restricted by the murine MHC protein.

Each of the T cell hybridomas that responded by proliferating when presented with the DRB1*0401 antigen was subcloned. The responses of each were shown to be inhibited by the monomorphic anti-DR antibody LB3.1 but not by the anti-I-A^f antibody 10.3.6.2 (Oi et al., *Current Topics in Microbiol. Immunol.* 81:165), as shown in Figure 2. The responses were measured in the presence of 10, 5, 2, and 0 µg/ml of antibody, showing a dose-dependent response to the antibody specific for the human MHC protein with complete inhibition at 10 µg/ml LB3.1.

The restriction of three of the T cell hybridomas in the context of several human DRB proteins was also investigated, as shown in Figure 5. T cell hybridomas B210, B136, and B45 recognized the peptide of SEQ ID NO:2 only in the context of the DRB1*0401 protein, and not in the context of the DRB1*0402, DRB1*0404, DRB1*0405, and DBR1*0101 human MHC class II proteins.

EXAMPLE II
COLLAGEN EPITOPE IMMUNODOMINANT FOR
PURIFIED HUMAN MHC DRB1*0401

5 The primary sequence of Bovine type II collagen was analyzed using an algorithm for predicting peptide binding to HLA DRB1 *0401 based on independent contributions of sidechains in the free energy of binding (see, Hill et al., *J. Immunology* 152:2890 (1994) and Marshall et al., *J. Immunology* 152:4946 (1994)).

10 The 25 peptides which were predicted to bind with the lowest IC₅₀ values (i.e. have the strongest binding to HLA DRB1*0401), which include the peptides represented by SEQ ID NO:2 and SEQ ID NO:3, are listed in Fig. 8 (see, USSN 08/300811, incorporated herein by reference for a discussion of IC₅₀ values). This series of 25 thirteen amino acid peptides are representative of portions of the mature bovine type II collagen protein.

15

 Peptides described herein including peptides represented by SEQ ID NO:2, SEQ ID NO:3 AND SEQ ID NO:4 were synthesized using solid phase techniques (Barany et al. (1979) in *The Peptides*, (Gross et al., eds.) Academic Press, New York, p. 1) either using an Applied Biosystems Peptide synthesizer or an Advanced Chemtech robotics system utilizing FastMOC chemistry with commercially available Wang resins, and Fmoc protected amino acids as previously described (Hill et al. (1994) *J. Immunol.* 152:2890).

20

 Peptides represented by SEQ ID NO:2 and SEQ ID NO:3 were assayed along with the other peptides listed in Fig 8 in a competitive inhibition assay for binding to affinity purified DRB1*0401 protein which had been recombinantly produced from Chinese hamster ovary (CHO) cells using known methods. Competitive inhibition assays were performed as described by Hill et al. Varying concentrations of the competitor peptide were incubated either overnight or for 4 hours at 37°C with constant concentrations of either biotinylated influenza hemagglutinin peptide (HA 306-318) or a rat myelin basic protein peptide (rMBP 90-102) and HLA-DRB1*0401 in PBS containing 1% octyl- β -D-glucopyranoside.

25

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The concentration of unlabelled peptide that prevented 50% of the labelled peptide from binding was the IC_{50} value. The IC_{50} values were not equated to equilibrium binding constants because of the known decomposition of MHC class II molecules during the course of the assay (Cubbon et al. Submitted in *J. Immunol.*). After incubation, DR-peptide complexes were transferred and captured by incubating for four hours on a 96-well microtiter plate initially coated with the LB3.1 antibody described above, and blocked with fetal calf serum. Excess peptide was removed by washing with PBS containing 20 μ M Tween 20 and 0.05% NaN_3 . The plates were treated with europium labelled streptavidin (Pharmacia) and incubated overnight. After washing, complexes were treated with 0.1 M acetate/phthalate buffer, pH 3.2, containing 0.1% Triton X-100, 15 μ M 2-naphthoyltrifluoroacetone and 50 μ M tri-N-octylphosphine oxide, which releases europium from streptavidin and forms a highly fluorescent micellar solution that can be measured by reading the fluorescence on a fluorometer.

The results of this assay indicate that of the 25 peptides tested in this experiment, the peptides of SEQ ID NO:2 and SEQ ID NO:3 were shown to compete most effectively with the influenza hemagglutinin and rat myelin basic protein peptides, for binding to the purified human MHC antigen (See, Fig. 8).

EXAMPLE III

COLLAGEN PEPTIDE SPECIFICITY OF HUMAN DRB1*0401 T CELL HYBRIDOMAS

The T cell hybridomas which were restricted for the human MHC DRB1*0401 protein also were assayed to determine the specific type II collagen peptide recognized by the human protein. The hybridomas were incubated with 20 μ M of each of the peptides tested in Example II, or with 100 μ g/ml of the mixture of cyanogen bromide fragments, using the DRB1*0401 EBV transformed B cell line (Preiss cells) as antigen presenting cells.

In these assays the ability to respond was measured by using an ELISA that measured interleukin-2. After 24 hours of incubating the hybridomas (2×10^4 cells/ml), Priess cells (5×10^4 cells/ml), and 20 μ M of a series of type II collagen peptide, 100 μ l of the supernatant was taken from each well and transferred to a corresponding 96 well plate, previously coated with anti-interleukin-2 monoclonal antibody (Pharmingen, San Diego, CA). After one hour of incubation, the plate was washed with phosphate buffered saline containing 0.05% Tween 20, and 100 μ l of biotinylated anti-interleukin-2 (1 μ g/ml) (Pharmingen, San Diego, CA) dissolved in phosphate buffered saline containing 1% bovine serum albumin was added. After one hour of incubation, the plate was washed and 100 μ l of europium labelled streptavidin (100 ng/ml) (LKB-Wallac, Sweden) dissolved in calcium/magnesium-free phosphate buffered saline containing 7 mg/liter diethylenetriaminepentaacetic acid (Sigma, St. Louis, MO) and 1 g/liter bovine serum albumin was added. After 30 minutes, the plate was washed and 100 μ l of Enhance solution (Wallac, Turku, Finland), 0.1 M acetate/phthalate buffer, pH 3.2, containing 0.1% Triton X-100, 15 μ M 2-naphthoyltrifluoroacetone and 50 μ M tri-N-octylphosphine oxide) was added. The resulting fluorescence was read in LKB-Wallac fluorescent plate reader. The fluorescent signal was normalized to an interleukin-2 standard titration curve.

Under the conditions described, 19 of 24 T cell hybridomas responded to the peptides of SEQ ID NO:2 and SEQ ID NO:3. The remaining T cell hybridomas had specificities that differed from the 25 peptides tested in Example II. When tested at a concentration of 20 μ M, the peptides of SEQ ID NO:2 and SEQ ID NO:3 elicited approximately equal responses from the T cell hybridomas. In a titration experiment, the peptide of SEQ ID NO:2 demonstrated a greater potency than the peptide of SEQ ID NO:3, as shown in Figure 3.

EXAMPLE IV ANALOGS

The peptide of SEQ ID NO:4 and shorter peptides contained within the peptide of SEQ ID NO:4 including peptides of SEQ ID NO:2 and SEQ ID NO:3

(see Fig. 9) were also tested for their ability to bind to the purified human MHC protein DRB1*0401 in the competitive inhibition assay set forth in Example II and for their ability to stimulate interleukin-2 secretion of the collagen-specific T cell hybridoma B210. The results of the competitive inhibition assay are summarized below and shown in Fig. 9, and the results of the B210 T cell hybridoma stimulation assay are shown in Figure 4.

The peptide of SEQ ID NO:4 bound to purified DRB1*0401 and stimulated hybridoma B210 to secrete interleukin-2 somewhat better than the peptide of SEQ ID NO:2. Removal of the N-terminal alanine from the peptide of SEQ ID NO:2 had no effect on the resulting peptide's ability to bind to the purified antigen or its ability to stimulate interleukin-2 secretion. Removal of both alanine and glycine from the N-terminus of the peptide of SEQ ID NO:2 resulted in a five-fold loss of both binding to purified antigen and stimulation of interleukin-2 secretion. Deletion of the alanine, glycine, and phenylalanine from the N-terminus of the peptide of SEQ ID NO:2 resulted in a dramatic loss of activity in both assays. Deletion of residues from the carboxyl terminus of the peptide of SEQ ID NO:2 resulted in loss of binding and ability to stimulate T cell hybridomas to secrete interleukin-2. Removal of the C-terminal glycine, glutamine, and proline resulted in an analog which bound similarly to the peptide of SEQ ID NO:3 and which stimulated interleukin-2 secretion similarly to that peptide. Removal of the C-terminal lysine, glycine, glutamine, and proline resulted in an analog which was able to bind the purified DRB1*0401 protein but which was unable to stimulate the T cell hybridoma to secrete interleukin-2. Removal of the C-terminal proline, lysine, glycine, glutamine, and proline resulted in an analog which was both unable to bind the purified DRB1*0401 protein and able to stimulate the T cell hybridoma to secrete interleukin-2 only weakly.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ImmuLogic Pharmaceutical Corporation

(ii) TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR
TREATING RHEUMATOID ARTHRITIS

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Lappin & Kusmer

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(F) ZIP: 02109

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Kerner, Ann-Louise

(B) REGISTRATION NUMBER: 33,523

(C) REFERENCE/DOCKET NUMBER: IMZ-014PCT

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-466-6000

(B) TELEFAX: 617-466-6040

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos taurus type II collagen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Pro Thr Gly Pro Leu Gly Pro Lys Gly Gln Thr Gly Glu Leu Gly Ile
1 5 10 15
—
Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys
 20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos taurus type II collagen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys Gly Glu Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos taurus type II collagen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos taurus type II collagen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys Gly Glu Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos Taurus type II collagen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Pro Arg Gly Pro Xaa Gly Pro Xaa Gly Pro Ala Gly Leu Xaa Gly
1 5 10 15

Pro Ser Gly Glu Xaa Gly Pro Lys
20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos taurus type II collagen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gly Glu Xaa Gly Ala Xaa Gly Pro Ala Gly Pro Xaa Gly Glu Xaa Gly
1 5 10 15

Ala Xaa Gly Pro Ala Gly Pro Xaa Gly
20 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bos taurus type II collagen

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Glu Glu Gly Leu Arg Gly Ala Arg Gly Glu Xaa Gly Glu Arg Gly
1 5 10 15

Pro Xaa Gly Pro Gln Gly Ala Arg
20

CLAIMS

1. A peptide comprising an amino acid sequence selected from the group consisting of the amino acid sequence set forth in SEQ ID NO:2, the amino acid
5 sequence set forth in SEQ ID NO:3, and the amino acid sequence set forth in SEQ ID NO:4, all as shown in Fig. 6.
2. A modified form of the peptide according to claim 1, wherein the modification
10 comprises substituting for one or more amino acids of said peptide a different amino acid which does not interfere with the biological activity of said peptide.
3. The modified peptide of claim 2, wherein the modification comprises replacing
15 at least one amino acid residue of said peptide which is essential to interact with the MHC with another similar amino acid.
4. The modified peptide of claim 2, wherein the modification comprises replacing
20 at least one amino acid residue of said peptide which is not essential for interaction with the MHC protein complex but which still bind the MHC protein complex with another amino acid selected from the group consisting of alanine, glutamic acid or a methyl amino acid.
5. A modified form of the peptide according to claim 1, wherein the modification
25 comprises an additional moiety coupled to said peptide, said moiety selected from the group consisting of polyethylene glycol, a moiety that enhances the solubility of the peptide, a moiety that facilitates purification of said peptide, and a moiety which comprises a proteolytic cleavage site.
6. The modified peptide of claim 5, wherein the modification comprises an
30 additional moiety coupled to said peptide, wherein said enhances the solubility of the peptide.

7. The modified peptide of claim 6, wherein the modification comprises the addition of at least one charged amino acid to said amino terminus, carboxy terminus or both the amino terminus and carboxy terminus of said peptide, said charged amino acid pairs being selected from the group consisting of arginine (R), lysine (k),
5 histidine (H), glutamic acid (E), and aspartic acid (D).
8. A therapeutic composition comprising at least one peptide selected from the group consisting of: the amino acid sequence set forth in SEQ ID NO:2, the amino
10 acid sequence set forth in SEQ ID NO:3, and the amino acid sequence set forth in
SEQ ID NO:4, all as shown in Fig. 6.
9. The therapeutic composition according to claim 8, further comprising at least one peptide selected from the group consisting of SEQ ID NO:1, SEQ ID NO:5, SEQ
15 ID NO:6, and SEQ ID NO:7, all as shown in Fig. 7.
10. A therapeutic composition comprising at least one modified peptide of claim 7, said modified peptide capable of down regulating an autoimmune response in rheumatoid arthritis.
- 20 11. A method of treating a mammal suffering from rheumatoid arthritis which comprises administering a therapeutically effective amount of at least one therapeutic composition of claim 8.
- 25 12. A method of treating a mammal suffering from rheumatoid arthritis which comprises administering a therapeutically effective amount of at least one therapeutic composition of claim 9.
- 30 13. A method of treating a mammal suffering from rheumatoid arthritis which comprises administering simultaneously or sequentially a therapeutically effective amount of at least two pharmaceutical compositions of claim 8.

14. A method of treating a mammal suffering from rheumatoid arthritis which comprises administering simultaneously or sequentially a therapeutically effective amount of at least two compositions of claim 9.

5 15. The method according to claim 11 wherein said administration is in non-immunogenic form by subcutaneous injection, intravenous injection, intramuscular injection, intraperitoneal injection, transdermal administration, oral administration, inhalation, aerosol, sublingual administration or enteral administration.

10 16. The method according to claim 12 wherein said administration is by subcutaneous injection, intravenous injection, intramuscular injection, intraperitoneal injection, transdermal administration, oral administration, inhalation, aerosol administration, sublingual administration and enteral administration.

15 17. The method according to claim 11 wherein said composition is administered subcutaneously in non-immunogenic form.

18. A method of treating a mammal suffering from rheumatoid arthritis comprising subcutaneously administering to a mammal, in non-immunogenic form, a
20 therapeutically effective amount of a composition comprising at least one peptide selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, all as shown in Fig. 7.

19. A peptide comprising the amino acid sequence set forth in SEQ ID NO:2.

25 20. A therapeutic composition comprising the peptide of claim 19 and a pharmaceutically acceptable carrier.

1/10

1. ZMGZGFBZKG AGAAMGVMGP MGPRGPPGPA GAPGQQGFQG NPGEPEPGV
51 SGPMGPRGPP GPPGKPGDDG EAGKPKSGE RGPPGPQGAR GFPGTPGLPG
101 VKGHRGYPLG DAKGEAGAP GVKGESGSPG ZBGSPGPMGP RGLPGERGRT
151 GPAGAAGARG NDGQPGPAGP PGPVGPAGGP GFPAPGAKG EAGPQGARGP
201 EGAQGPGRGEP GTPGAPGPAG AAGNPGADGI PGAAGSAGAP GIAGAPGFPG
251 ARGPPGPTGA SGPLGPKGQT GEPGIAGFKG EQGPKGEPGP AGVQAGPAPA
301 GEEGKRGARG EPGGAGPAGP PGERGAPSR GFPQDGIAG PKGPPGERGS
351 PGAVGPKGSP GEAGRPGAG LPGAKGLTGR PGDAGPQKV GPSGAPGEDG
401 RPPGPPQGA RGQPGVMGFP GPKGANGEPG KAGEKGLPGA PGLRGLPGKD
451 GETGAAGPPG PAGPAGERGE QGAPGPSGFQ GLPGPPGPPG EPTLDAMKVF
501 CNMETGETCV YPNPASVPKK NWWSSKSKDK KHIWFGETIN GGFHFSYGDD
551 NLAPNTADVQ MTFRLRLSTE GSONITYHCK NSIAYLDEAA GNLKKALLIQ
601 GSNDVEIRAE GNSRFTYTVL KDGCTKHTGK WGMTMIEYRS QKTSRLPIID
651 IAPMDIGGPE QEFGVDIGPV CFL

FIG. 1

2/10

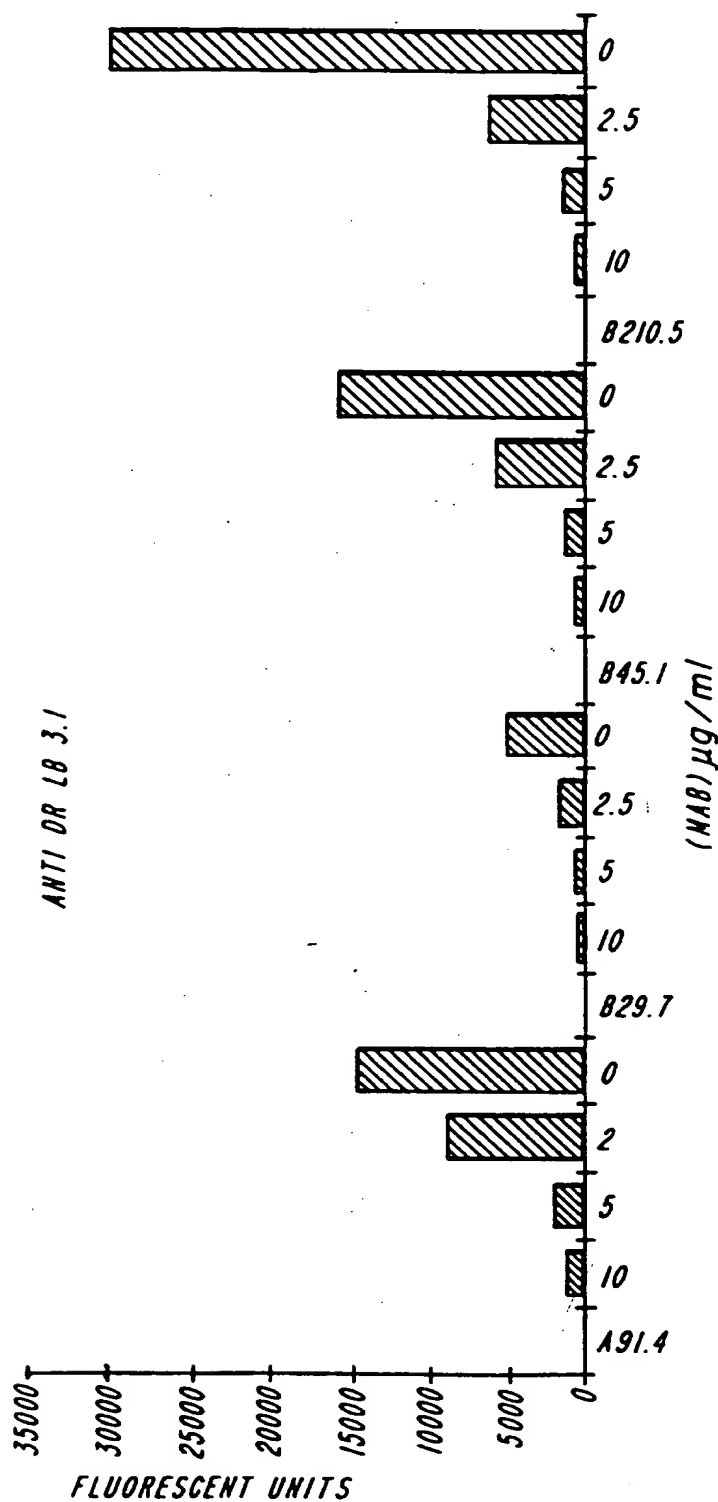


FIG. 2A

3/10

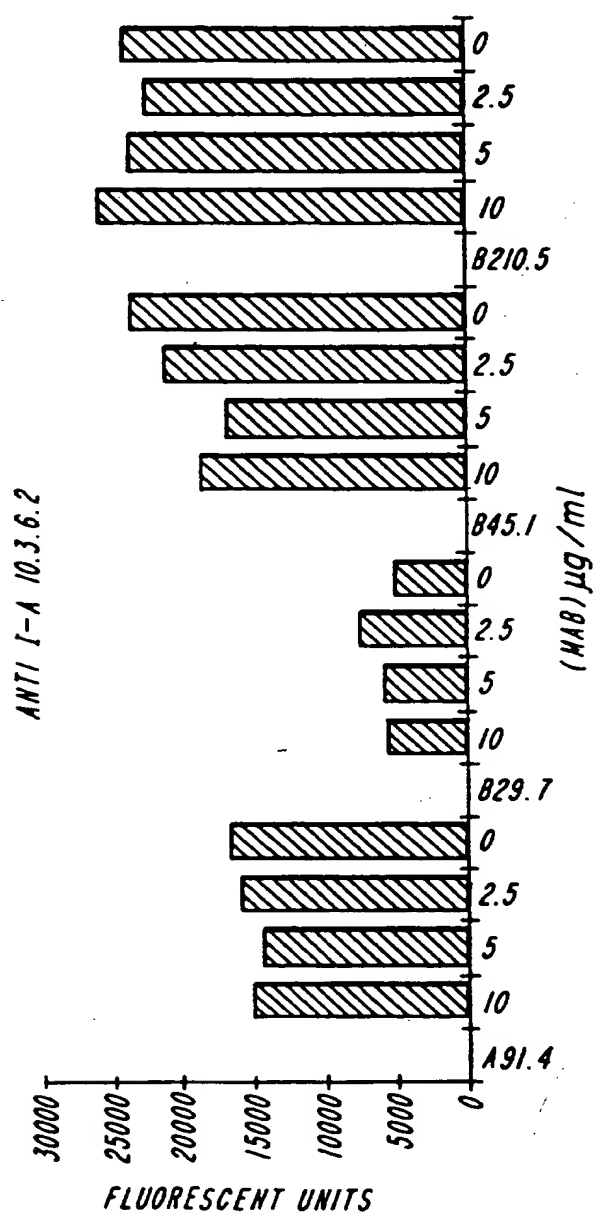
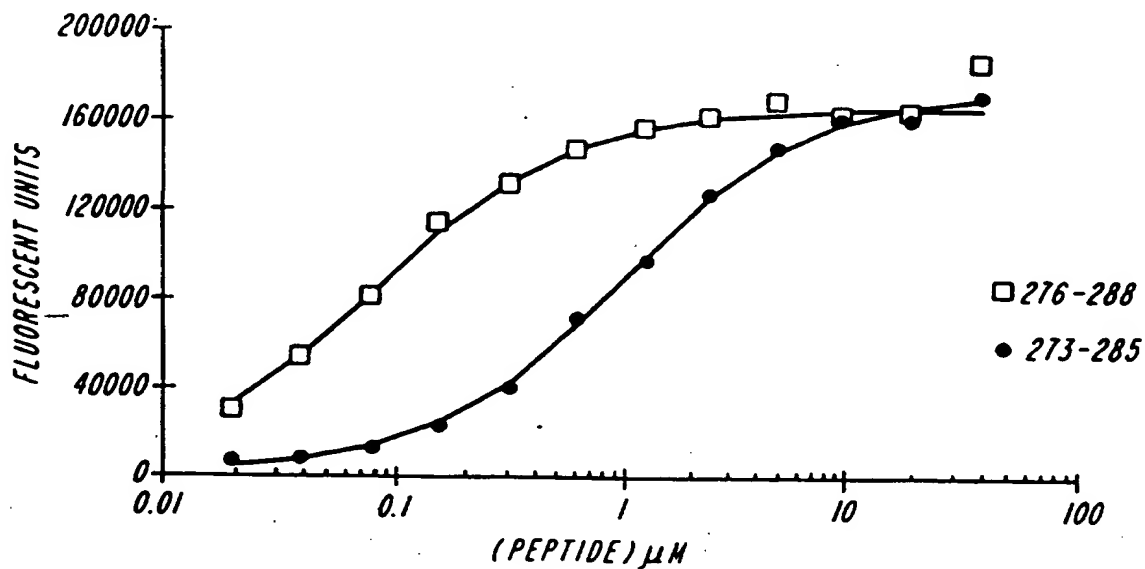
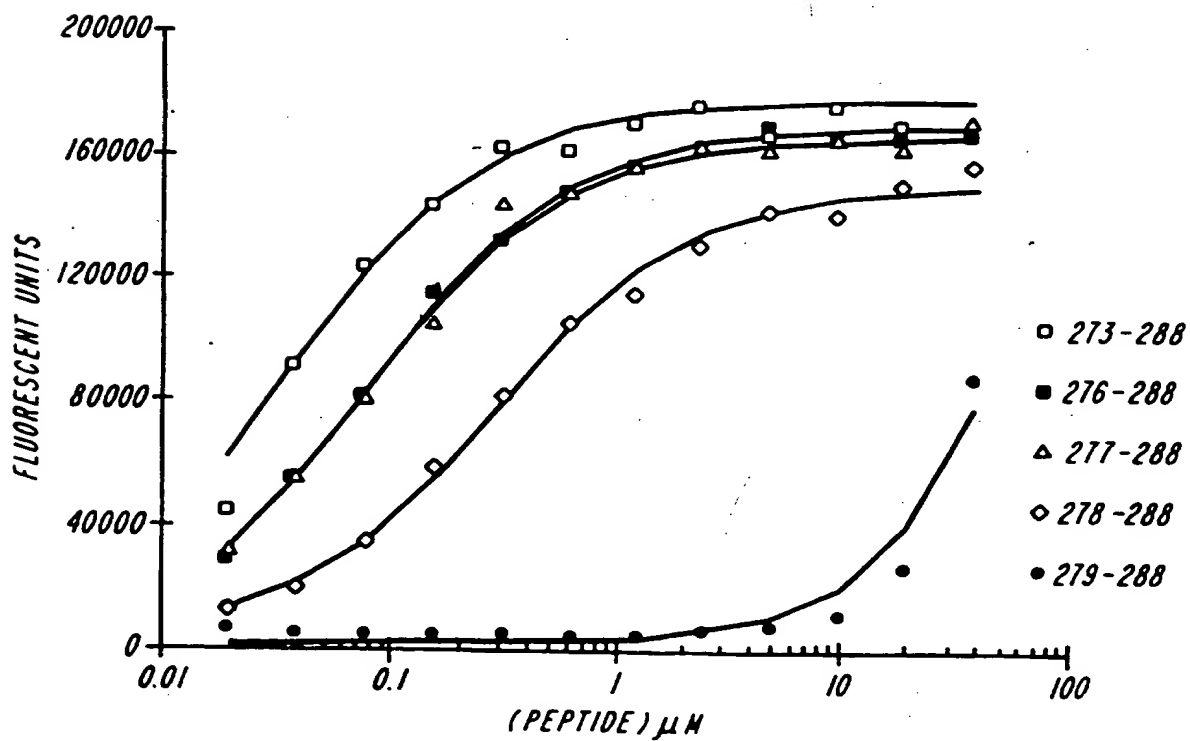
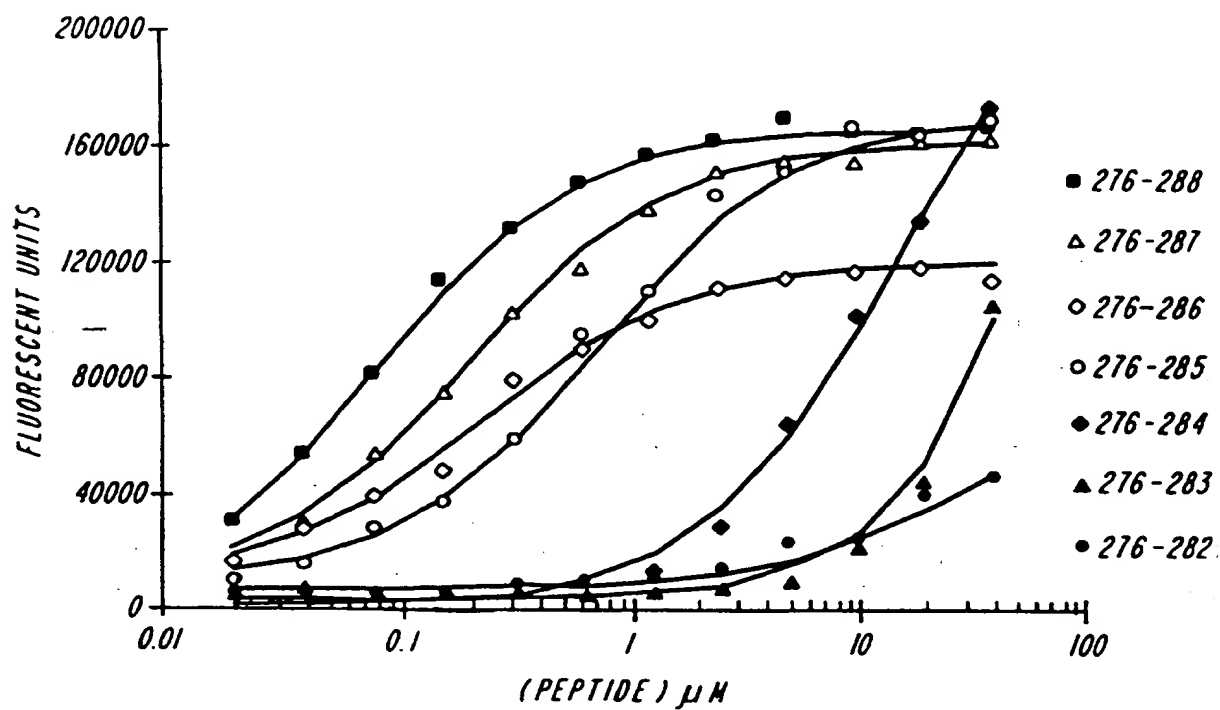


FIG. 2B

4/10

**FIG. 3****FIG. 4A**

5/10

**FIG. 4B**

6/10

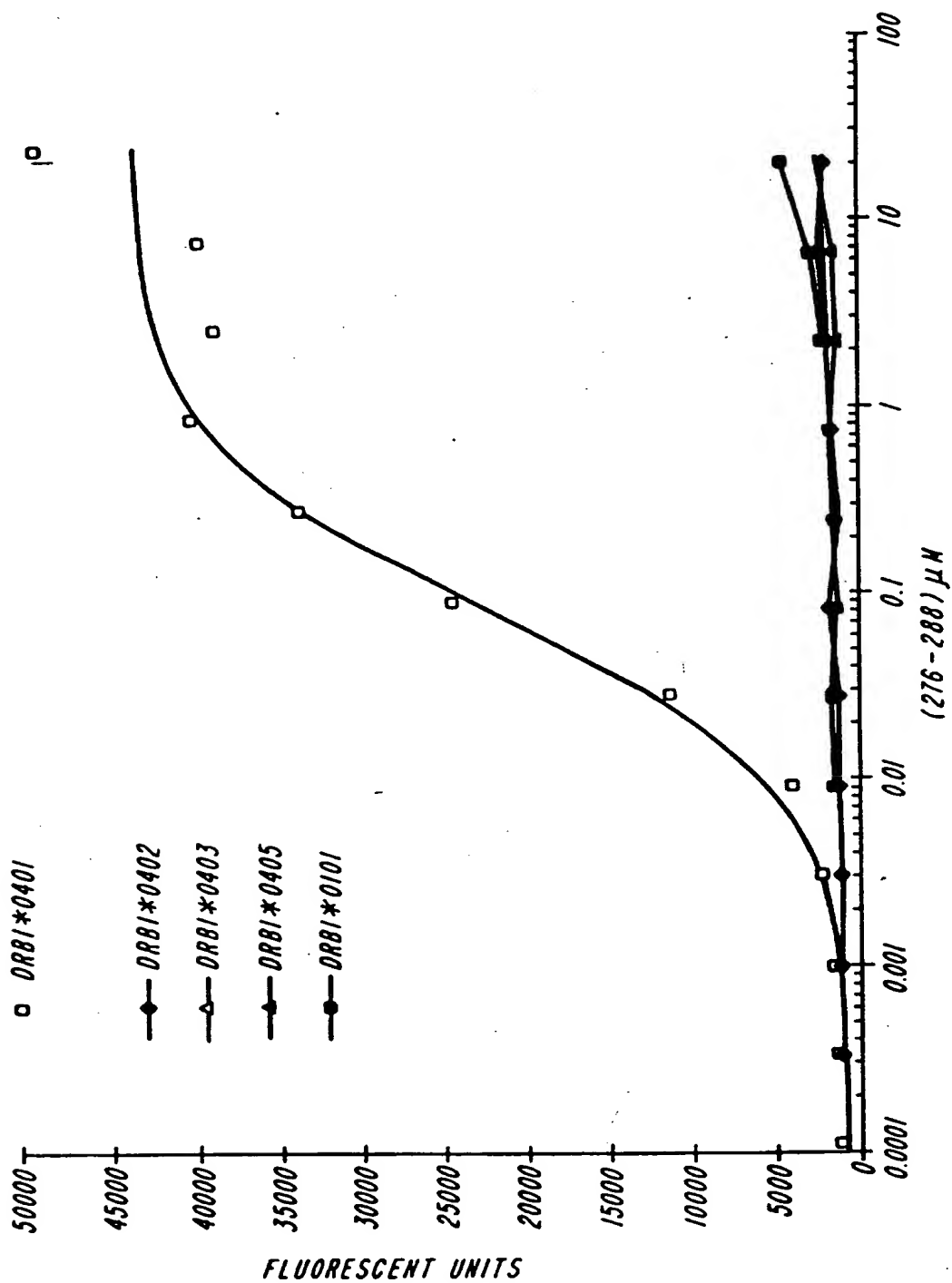


FIG. 5

7/10

SEQ ID NO:2	Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys Gly Glu Pro	1 5 10
SEQ ID NO:3	Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys	1 5 10
SEQ ID NO:4	Pro Gly Ile Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys Gly Glu Pro	1 5 10 15

FIG. 6

8/10

SEQ ID NO:1 Pro Thr Gly Pro Leu Gly Pro Lys Gly Gln Thr Gly Glu Leu Gly Ile
1 5 10 15

Ala Gly Phe Lys Gly Glu Gln Gly Pro Lys
20 25

SEQ ID NO:5 Gly Pro Arg Gly Pro Xaa Gly Pro Xaa Gly Pro Ala Gly Leu Xaa Gly
1 5 10 15

Pro Ser Gly Glu Xaa Gly Pro Lys
20

SEQ ID NO:6 Gly Glu Xaa Gly Ala Xaa Gly Pro Ala Gly Pro Xaa Gly Glu Xaa Gly
1 5 10 15

Ala Xaa Gly Pro Ala Gly Pro Xaa Gly
20 25

SEQ ID NO:7 Gly Glu Glu Gly Leu Arg Gly Ala Arg Gly Glu Xaa Gly Glu Arg Gly
1 5 10 15

Pro Xaa Gly Pro Gln Gly Ala Arg
20

FIG. 7

9/10

RESIDUE NUMBER	HIERARCHY													HIERARCHY		HIERARCHY		MURINE
	PREDICTED													16 HR. HA	4 HR. RM8P			
	1	2	3	4	5	6	7	8	9	10	11	12	13	IC50(nM)	IC50(nM)	IC50(nM)		
246	P	G	F	K	G	A	R	G	P	P	G	P	T	6	8175	3	457	3
258	P	G	F	K	G	E	Q	G	E	K	G	E	P	8.4	885	1	182	1
276	A	G	F	K	G	H	R	G	P	P	G	L	D	12.0				0, T
99	P	G	V	K	G	L	P	G	P	P	G	P	P	18.7	6800	2	461	4
477	S	G	F	Q	G	N	P	G	E	P	G	E	P	59.3				
36	Q	G	F	Q	G	F	K	G	E	Q	G	P	K	79.2	19650	6	404	2
273	P	G	I	A	G	E	S	G	S	P	G	E	N	94.6	>100000	11	9380	10
120	P	G	V	K	G	F	P	G	P	K	G	A	N	155	30600	8	9370	9
414	P	G	V	M	G	F	P	G	F	P	G	A	N	290	14100	5	3150	6
240	P	G	I	A	G	A	P	G	P	P	G	A	R	463	29665	7	2415	5
408	Q	G	A	R	G	Q	P	G	V	M	G	F	P	665				P
291	A	G	V	Q	G	A	P	G	P	A	G	E	E	2454	57650	9	9290	8
13	A	A	M	G	V	M	G	R	M	G	P	R	G	5049				
441	P	G	L	R	G	L	P	G	K	D	G	E	T	5927	11850	4	9175	7
48	P	G	V	S	G	P	M	G	P	R	G	P	P				48050	11
336	D	G	I	A	G	P	K	G	P	P	G	E	R	7903	64950	10		
186	P	G	A	K	G	E	A	G	P	Q	G	A	R	7687	>1000000			T
474	P	G	P	S	G	F	Q	G	L	P	G	P	P	12406	>1000000			
408	Q	G	A	R	G	Q	P	G	V	M	G	F	P	13446	>1000000			M
87	Q	G	A	R	G	Q	P	G	T	P	G	L	P	14257	>1000000			
111	D	G	A	K	G	E	A	G	A	P	G	V	K	24540	>1000000			
33	P	G	P	Q	G	F	Q	G	N	P	G	E	P	34128	>1000000			S, N
204	Q	G	P	R	G	E	P	G	T	P	G	A	P	36980	>1000000			
27	P	G	P	A	G	A	P	G	P	Q	G	F	Q	39763	>1000000			Q, T
255	P	G	P	T	G	A	S	G	P	L	G	P	K	59275	>1000000			S, T
216	P	G	P	A	G	A	A	G	N	P	G	A	D	64971	>1000000			

FIG. 8

10/10

4HR (RMBP) 16 HR. (HA)

	1	2	3	4	5	6	7	8	9	10	11	12	13	
273 285	P	G	I	A	G	F	K	G	E	Q	G	P	K	404
276 288		A	G	F	K	G	E	Q	G	P	K	G	E	885
273 288	P	G	I	A	G	F	K	G	E	Q	G	P	K	104
276 287		A	G	F	K	G	E	Q	G	P	K	G	E	191.5
276 286		A	G	F	K	G	E	Q	G	P	K	G		193
276 285		A	G	F	K	G	E	Q	G	P	K			369
276 284		A	G	F	K	G	E	Q	G	P				394.5
276 283		A	G	F	K	G	E	Q	G					>100000
276 282		A	G	F	K	G	E	Q						>100000
276 281		A	G	F	K	G	E							>100000
276 288		A	G	F	K	G	E	Q	G	P	K	G	E	3755
277 288		A	G	F	K	G	E	Q	G	P	K	G	E	8495
278 288				F	K	G	E	Q	G	P	K	G	E	13365
279 288				K	K	G	E	Q	G	P	K	G	E	74050
HA 307 319		P	K	Y	V	K	Q	N	T	L	K	L	A	20

FIG. 9

